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Effects of amino acid sequence changes on antibody-antigen interactions

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Amino acid substitutions in antibody-antigen interfaces play an important role in affinity maturation of antibody responses and in antigenic variation. Structural studies show that some non-conservative changes are tolerated in these interfaces. By analogy with observations of amino acid exchangeability within homologous or mutated protein structures, this suggests that the number of different antibody specificities is less than the total number of antibodies which can be encoded by the various genetic mechanisms. On the other hand, conformational adaptability of antibody structures during binding of antigen suggests that one antibody can serve to bind a number of different antigens.

Single amino acid sequence changes within the interface of an antibody-antigen complex are important in two biological contexts. Firstly, such changes in the antibody have the capacity to drive the affinity towards more tightly bound complexes. Secondly, such changes in the antigen can effectively abolish the interaction entirely, providing an effective mechanism for antigenic variation. Although antibody and antigen display biologically asymmetric behaviour in regard to the effects of single amino acid substitutions, in principle and in practice, substitutions in either partner can raise or lower the affinity. Tolerance of amino acid sequence substitutions within antibody-antigen interfaces suggests that they

be considered examples either of protein-protein interactions generally or of the core of folded protein structures. In those cases, degeneracy of amino acid sequence information is well documented among families of homologous proteins, and it suggests that the number of different antibody specificities is less than the estimated number of different antibody molecules.

Antigenic variation

Substitutions which result in loss of binding are not amenable to direct study of complexes, although several studies have sought to rationalize the effects of the new amino acid in the context of the complex involving the "parent" amino acid.

Escape mutants of viral antigens, selected by growth of virus in the presence of monoclonal antibody, provide many examples of the type of substitution which can render the antigen unrecognizable by the selecting antibody (see, for example, Webster *et al.*, 1987). There are no discernible trends in these data which suggest preference for replacement of residues in one physico-chemical class by those in another as a preferred means of abolishing binding (Colman, 1992). Studies of the three-dimensional structures of a number of these antibody selected mutants shows that local changes, at and immediately adjacent to the site of the mutated residue, suffice to disrupt the interaction with the selecting antibody (Knossow *et al.*, 1984; Varghese *et al.*, 1988; Tulip *et al.*, 1991).

Tulip (1990) has observed that a variety of escape mutants of the influenza virus neuraminidase, selected by the anti-neuraminidase antibody NC41, result in shape changes of the antigen which could interfere with surface docking if the remainder of the antigen and the antibody were rigid and unable to relax around the mutated residue. In another case, a hydrogen bond is potentially lost by substitution to a smaller residue (Tulip, 1990). Bhat *et al.* (1990) report that substitution of Gln by His in lysozyme also causes shape changes which cannot be accommodated by the D1.3 anti-lysozyme antibody, and that, in addition, a hydrogen bond would be lost from the interaction. In both of these examples, the key to the failure of the mutant to bind may reside as much in the inflexibility of the surrounding structures as it does in the physico-chemical environment within the interface of the mutated residue.

Structural data on the effects of point changes in the binding site are available in only one system at present, the influenza virus neuraminidase (Tulip *et al.*, 1992). Two mutants of the N9 subtype neuraminidase, selected with antibodies other than NC41, involve substitutions within the binding site on N9 for NC41. In each case, the affinity is reduced by one to two orders of magnitude (Webster *et al.*, 1987; Gruen *et al.*, 1993). One of these mutants, Asn 329 to Asp, is located near the edge of the binding site for the NC41 antibody and is accommodated by re-positioning the side chain of residue 329 towards the solvent exposed perimeter of the antibody-antigen interface. The other, Ile 368 to Arg, results in a complex pattern of concerted movements around the mutation site to accommodate the arginyl residue. These changes include a shift by 3Å in the position of the arginine from its location in the uncomplexed neuraminidase structure and a shift by more than 1Å of a histidine on the antibody. In both of these structures, the effects of the mutations within the interface are reminiscent of the effects of amino acid substitutions within the interior of protein molecules (Matthews, 1991), Anderson *et al.* (1993) where localized structural rearrangements around the mutation site are frequently observed. Analysis of structures in the Brookhaven data base (Bernstein *et al.*, 1977) suggests a somewhat looser packing density of atoms within an antibody-antigen interface compared to other protein-protein interfaces or the interior of protein molecules (Tulip *et al.*, 1992; Lawrence and Colman, 1993). This suggests, in turn, that antibody-antigen interfaces should not be more sensitive to amino acid substitutions than other protein-protein interfaces.

Somatic mutation

Point mutations accumulating within the variable domains of antibody heavy and light chains are associated with increasing affinity of the antibody for antigen.

Alzari *et al.* (1990) have demonstrated that when 2-phenyloxazolone binds to an antibody, it makes direct contact with amino acids of the V_L domain which are known to be frequently mutated in antibodies with increased affinity for the antigen. In contrast, in this same system, somatic mutation in the V_H domain in no case maps to amino acids directly involved in binding the hapten.

Engineered substitutions in an anti-*p*-azophenylarsonate antibody (Sharon, 1990) have demonstrated that three of nineteen somatic changes observed in the V_H domain suffice to increase the affinity by a factor of 200. Comparison with the three-dimensional structure of the antibody (Rose *et al.*, 1990) suggests that, in every case, the effect must be indirect, since none of the three substituted residues is likely to be in direct contact with the antigen. In this example, there is not yet a report of the antibody-antigen complex structure, nor is the antibody structure highly refined, so the conclusion should be considered preliminary.

Structural context and repertoire size

The above examples paint a confusing picture of the specificity of antibody-antigen interaction. In one structural context, a very conservative substitution may abolish binding; in another, a non-conservative substitution may have very little effect on the binding affinity. How should these observations be treated if one is to estimate the effective size of the antibody repertoire from genetic sources alone? One approach is to treat the antibody-antigen interface the same way as the interior of a protein structure, and to bring to bear on this problem current knowledge about the degeneracy of "structural information" among the twenty amino acids.

Current estimates of the potential number of antibody molecules that can be generated by all the known genetic mechanisms is in excess of 10^{18} (Hunkapiller and Hood, 1989). This and similar other estimates assume each of the 20 amino acids is different from every other amino acid, which is appropriate for purposes of enumeration but not for the purpose of estimating how many different antibody specificities can be produced by an animal.

Data from exchangeability matrices (Dayhoff *et al.*, 1978) or from degeneracy of information in amino acid sequences resulting from overlapping genes (Sander and Schulz, 1979) suggest that, for structural purposes, or more precisely for folding purposes, there are effectively only four or five "different" amino acids. Can the same reduction be applied to binding interactions between proteins? Folding could be considered to be more tolerant of amino acid substitutions because of the cooperative effects caused by the interactants being covalently linked to each other. Binding interactions could be considered less tolerant because the changes involved occur in what might be called the active site. Outside of the antibody-antigen system, mapping of protein-protein interacting surfaces by mutational analysis is also generally successful (Bowie *et al.*, 1990), but some unexpected and unexplained findings do occur (de Voss *et al.*, 1992), suggesting a measure of tolerance of amino acid substitutions generally in protein-protein interfaces.

These arguments affect considerations of antibody repertoire sizes. In the facile extreme of substituting five (types of amino acids) for twenty in the calculations of Hunkapiller and Hood (1989), the expected number of different antibody specificities is reduced to order 10^8. Another way to estimate the number of different specificities is to argue from the physical size of a typical binding site on antibody for antigen. For protein antigens, this surface size is of the order of 15 amino acids. The numbers of different specificities that can be encoded over such a surface based on twenty or five structurally different amino acids are order 10^{19} and 10^{10}, respectively, if one ignores the important influences of overall surface shape and partiality of solvent exposure of amino acids within the surface. Similar arguments and estimates of repertoire size are applicable to T-cell receptors.

Some compromise in the capacity of the immune system to cope with "foreign" structures is implied by these degeneracy arguments. The above estimates have carried the argument to the extreme and could therefore be viewed as an extreme lower limit of repertoire size. Nevertheless, genetic sources of diversity are only part of the story. Antibodies, as proteins, display the usual types of conformational adaptability in binding to ligands, as do other proteins, *i.e.* side chain rearrangements and main chain changes within loop structures (Colman, 1988; Wilson and Stanfield, 1993). In addition, there is growing experimental evidence for the functioning of the V_L-V_H interface as a structural adaptor allowing movements of the heavy chain CDR *en masse* with respect to the light chain CDR during engagement with antigen (Colman, 1988, 1991; Herron *et al.*, 1991; Bhat *et al.*, 1990; Stanfield *et al.*, 1993). These changes in antibody structure are believed to be specific to the interacting antigen. Different antigens may therefore induce different structural responses in the same antibody, adding a structural dimension to diversity (Colman, 1988).

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Recognition of carbohydrates by antibodies

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The recognition by antibodies of complex polysaccharides forming the outer layer of cell walls is an integral part of the immune response to bacterial infection and invasion by non-self cells. For these reasons, the interactions of antibodies with sugars have been extensively investigated by physicochemical and immunochemical methods (Young *et al.*, 1983; Bundle, 1989; Glaudemans, 1991; Sigurskjold and Bundle, 1992). The conformation of free (Bush, 1992) and antibody-bound oligosaccharides has been studied by NMR spectroscopy (Glaudemans, 1991; Bundle *et al.*, submitted) and crystallography (Cygler *et al.*, 1991; Vyas *et al.*, 1993). NMR measurements showed that in some cases, binding to an antibody